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Population structure of invasive *Streptococcus pneumoniae* isolates among Alaskan children in the conjugate vaccine era, 2001 to 2013☆,,☆☆

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Abstract

Here we describe the relationships between serotypes, genotypes, and antimicrobial susceptibility among isolates causing invasive pneumococcal disease in Alaskan children during the pneumococcal conjugate vaccine (PCV) era. From 2001 to 2013 we received 271 isolates representing 33 serotypes. The most common serotypes were 19A (29.5%, *n*= 80), 7F (12.5%, *n*= 34), 15B/C (6.3%, *n*= 17), and 22F (4.8%, *n*= 13). Multilocus sequence typing identified 11 clonal complexes (CC) and 45 singletons. Five CCs accounted for 52% (141/271) of the total: CC199 (21% [*n*= 57], serotypes 19A, 15B/C), CC191 (12.2% [*n*= 33], serotype 7F), CC172 (10.3% [*n*= 28], serotypes 19A, 23A, 23B), CC433 (4.4% [*n*= 12], serotype 22F), and CC100 (4.4% [*n*= 12], serotype 33F). The proportion of isolates nonsusceptible to erythromycin and tetracycline increased after 13-valent PCV use (14% [*n*= 30] versus 29% [*n*= 14]; *P*= 0.010) and (4% [*n*= 9] versus 22% [*n*= 11]; *P*< 0.001), respectively. The genetic diversity also increased after 13-valent PCV use (Simpson's diversity index =0.95 versus 0.91; *P*= 0.022).

Keywords

Streptococcus pneumoniae; Pneumococcal conjugate vaccines; Molecular epidemiology; Multilocus sequence typing; Antimicrobial susceptibility; Serotypes

1. Introduction

Worldwide each year, there are over 14 million serious *Streptococcus pneumoniae* (*S. pneumoniae*) infections in children <5 years of age leading to over 800,000 deaths (O'Brien

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Conflict of Interest

The authors have no conflict of interest to disclose.

et al., 2009). There are at least 95 capsular serotypes, but only a few cause the majority of disease. In 2001, the United States began 7-valent pneumococcal conjugate vaccine (PCV7) use in children <2 years of age. This vaccine contains polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. These serotypes caused approximately 80% of invasive pneumococcal disease (IPD) in US infants at that time (Robinson et al., 2001). After PCV7 use, many countries documented a decrease in PCV7-serotype IPD rates; however, increases in non–PCV7-serotype IPD were also documented in many of these same countries (Whitney et al., 2003; Hicks et al., 2007; Tyrrell et al., 2009; Oftadeh et al., 2013; Maraki et al., 2010; Isaacman et al., 2010; Ben-Shimol et al., 2014). This serotype shift led to the 2010 US implementation of a 13-valent pneumococcal conjugate vaccine (PCV13) for children <2 years of age. PCV13 includes PCV7 serotypes as well as 6 additional (PCV6; serotypes 1, 3, 5, 6A, 7F, and 19A). Data from many parts of the world, including the United States, show that PCV13 is decreasing IPD caused by these additional serotypes (Ben-Shimol et al., 2014; Richter et al., 2014; Waight et al., 2015; Moore et al., 2015; Cohen et al., 2016).

Alaska IPD rates have historically been among the highest in the world (Davidson et al., 1994). Alaska began using PCV7 in 2001 with vaccine rollout being rapid and fairly consistent throughout the state. As was seen elsewhere, PCV7 use in Alaska resulted in a change in the serotypes causing IPD. In children <2 years of age, the PCV7-serotype IPD rate decreased 96% and the non–PCV7-serotype IPD rate increased 140% (Singleton et al., 2007). PCV13 replaced PCV7 in the Alaska vaccination schedule in April 2010. PCV13 rollout was more rapid in one region as compared with the rest of the state due to an existing PCV13 clinical trial in that region (Singleton et al., 2013). In children <5 years of age, the overall IPD rate in Alaska decreased 59% from 2010 to 2013 as compared with 2005 to 2008 (Bruce et al., 2015).

Pneumococcal conjugate vaccines (PCVs) target the serotype-specific capsular genes; however, it is known that pneumococci include different clones that can express the same capsular gene (Henriques-Normark et al., 2008). Therefore, relying on serotype data alone gives only partial information about the pneumococcal population structure. Multilocus sequence typing (MLST) is now commonly used to better understand pneumococcal epidemiology (Enright & Spratt, 1998). In this study, we used MLST to characterize pneumococci causing IPD in young Alaskan children and to describe relationships between serotypes, antimicrobial nonsusceptibility, and MLST clonal complexes (CC) and sequence types (ST). We also investigated changes in the pneumococcal diversity.

2. Materials and methods

2.1. Bacterial isolates

Since 1986, *S. pneumoniae* isolated from a normally sterile site in an Alaska resident are sent from laboratories around the state to the US Centers for Disease Control and Prevention's Arctic Investigations Program (AIP) in Anchorage (Bruce et al., 2015). The AIP laboratory confirms all pneumococci using standard methods (Ruoff et al., 1999). Isolates are serotyped using latex agglutination and the Quellung reaction with group-specific and type-specific antisera (Staten Serum Institute, Copenhagen, Denmark) (Austrian, 1976).

2.2. Antimicrobial susceptibility testing

The AIP laboratory performs all susceptibility testing. Broth microdilution is used to determine minimum inhibitory concentrations for penicillin (PEN), erythromycin (ERY), trimethoprim-sulfamethoxazole (SXT), tetracycline (TET), and cephalosporins (cefotaxime/ceftriaxone [CTX/CRO]) (Clinical and Laboratory Standards Institute, 2007). Per Gertz et al., nonsusceptibility interpretation is made using the 2007 interpretive Clinical and Laboratory Standards Institute guidelines and includes isolates that are intermediate or fully resistant to a particular antibiotic (Clinical and Laboratory Standards Institute, 2007; Gertz et al., 2010). An isolate is considered multidrug resistant (MDR) if it has intermediate or full resistance to 3 or more antibiotic classes.

2.3. Multilocus sequence typing

For this study, we completed MLST on pneumococcal isolates received from children <5 years of age collected from 2001 to 2013. To extract DNA, we transferred *S. pneumoniae* cells to 100 μL nuclease-free water. The suspensions were vortexed for 10 to 15 seconds, heated at 100 °C for 10 minutes, and centrifuged at 13,000 rpm for 5 minutes. The supernatant was removed and stored at −30 °C until use. We performed MLST as previously described (Enright & Spratt, 1998) determining the STs by comparing our sequences with those from the *S. pneumoniae* MLST database (http://pubmlst.org/spneumoniae). For alleles or allelic profiles not in the MLST database, we submitted the trace files or allelic profiles to the MLST database curator for new ST assignments. We assigned CCs using the eBURST algorithm (http://eburst.mlst.net) and the stringent definition of 6/7 identical loci.

2.4. Statistical analysis

We compared isolate proportions by serotype, ST, and additional factors using chi-squared or Fisher's exact test as appropriate. We defined time periods to correspond with Alaska's vaccine introduction: 2001 to 3/2010 "PCV7 era", and 4/2010 to 2013 "PCV13 era". Incidence rates were calculated using population estimates obtained from the State of Alaska, Department of Labor (http://laborstats.alaska.gov/pop/popest.htm) and were compared using the statistics described in Rothman (Rothman, 1986). We measured diversity using the Gini-Simpson Index which estimates the probability that 2 randomly drawn isolates are different types (Heip & Engels, 1974) and compared diversity indices using a Student's *t* test as described in Lande (Lande, 1996). *P* values are two-sided and a *P* value <0.05 is considered statistically significant. Analyses were conducted in Stata Ver10.

3. Results

3.1. Bacterial isolates

From 2001 to 2013, the AIP received 271 pneumococcal isolates from Alaska residents <5 years of age with IPD. Isolates came from blood (n= 252), cerebrospinal fluid (n= 22), pleural fluid (n= 9), and other sterile sites (n= 8). Twenty cases had 2 isolates (blood/cerebrospinal fluid, n= 18; blood/pleural fluid, n= 2), but they were identical to each other so they were considered as one for this analysis.

3.2. Serotype distribution

Overall, we identified 33 serotypes among the 271 IPD isolates (Fig. 1). There were no serotype differences between the isolate sources and for serotypes for which there was a large enough sample size, serotypes were distributed across the state. Four serotypes accounted for over 53% (144/271) of the isolates: 19A (29.5%, *n*= 80), 7F (12.5%, *n*= 34), 15B/C (6.3%, *n*= 17), and 22F (4.8%, *n*= 13). In the PCV7 era, 3 serotypes covered over 51% (114/222) of the isolates: 19A (32.4%, *n*= 72), 7F (14.4%, *n*= 32), and 15B/C (4.5%, *n*= 10). In the PCV13 era, 5 serotypes covered over 55% (27/49) of isolates: 19A (16.3%, *n*= 8), 15B/C (14.3%, *n*= 7), 12F (8.2%, *n*= 4), 15A (8.2%, *n*= 4), and 22F (8.2%, *n*= 4).

Despite only 1 PCV7 serotype (19F) identified in the PCV13 era, the proportion of PCV7 serotypes did not change from the PCV7 era to the PCV13 era (10.4% [23/222] versus 4.1% [2/49]; *P*= 0.27; Fig. 2). The proportion of PCV6 serotypes decreased from 54% (120/222) in the PCV7 era to 20% (10/49) in the PCV13 era (*P*< 0.001). All PCV6 isolates in the PCV13 era were serotypes 19A (*n*= 8) and 7F (*n*= 2). The PCV6-IPD rate also decreased during this time period (25.5 versus 4.85 per 100,000; *P*< 0.001).

We identified 22 nonvaccine type (NVT) serotypes. Five serotypes accounted for 53% (62/116) of the NVT isolates: 15B/C (14.7%, *n*= 17), 22F (11.2%, *n*= 13), 12F (10.3%, *n*= 12), 33F (10.3%, *n*= 12), and 15A (6.9%, *n*= 8). Although the NVT-IPD rate did not change (16.79 versus 17.93 per 100,000; *P*= 0.733), the proportion of NVT serotypes increased from 36% (79/222) in the PCV7 era to 76% (37/49) in the PCV13 era (*P*< 0.001). NVT serotypes contributing to this increase were serotypes 15B/C (4.5% [10/222] versus 14.3% [7/49]; *P*= 0.019), 15A (1.8% [4/222] versus 8.2% [4/49]; *P*= 0.038), and 35B (0.9% [2/222] versus 6.1% [3/49]; *P*= 0.043). IPD rates did not change for any NVT serotype.

3.3. MLST

Overall, we identified 83 STs; 18 were new to the MLST database. ST199 (serotypes 19A [*n*= 32] and 15B/C [*n*= 8]) was the most common; 44 STs had only 1 isolate. Two STs, ST644 and ST3934, were associated with serotype 15B/C which had not previously been documented in the MLST database. Of the 83 STs, 52 were found only during the PCV7 era (Fig. 3; black), 16 were found only during the PCV13 era (green), and 15 were found in both eras (pink). A majority of isolates from the PCV13 era (63% [31/49]), were associated with STs that were circulating in the PCV7 era.

The 83 STs resolved into 11 CCs and 45 singletons (Table 1). PCV7 serotypes were represented by 14 CCs/STs, PCV6 serotypes by 12 CCs/STs, and NVT serotypes by 33 CCs/STs. The genetic diversity within PCV7 (Simpson's diversity index [SDI]= 0.94) and NVT serotypes (SDI = 0.95) was significantly higher than the genetic diversity within PCV6 serotypes (SDI = 0.77; *P*< 0.001 for both PCV7 versus PCV6 and NVT versus PCV6). Five CCs accounted for 52% (142/271) of the isolates: CC199 (21% [*n*= 57]; serotypes 19A, 15B/C), CC191 (12.2% [*n*= 33]; serotype 7F), CC172 (10.3% [*n*= 28]; serotypes 19A, 23A, 23B), CC433 (4.4% [*n*= 12]; serotype 22F), and CC100 (4.4% [*n*= 12]; serotype 33F). CC199 was the most common CC/ST in both the PCV7 (22.1%, *n*= 49) and PCV13 (16.3%, *n*= 8) eras. In the PCV13 era 50% (4/8) of CC199 isolates were associated with serotype

19A. This is a nonsignificant decrease from 83.7% (41/49; P= 0.052) in the PCV7 era. The remaining CC199 isolates in both eras were serotype 15B/C. CC172 and ST63 (serotype 15A) were the only CCs/STs to show significant changes in proportion from the PCV7 era to the PCV13 era. The CC172 proportion decreased from 12.2% (n= 27) to 2% (n= 1; P= 0.037). Serotype 19A accounted for 26/27 (96%) CC172 isolates in the PCV7 era and serotype 23A, collected during the first week of PCV13 use, was the lone CC172 isolate in the PCV13 era. The ST63 proportion increased from 0.5% (n= 1) in the PCV7 era to 8.2% (n= 4; P= 0.004) in the PCV13 era. The population's genetic diversity increased during the PCV13 era as compared with the PCV7 era (SDI = 0.95 versus 0.91; P= 0.022).

3.4. Antimicrobial susceptibility

Table 2 shows the proportion of isolates nonsusceptible to the 5 antibiotics of interest. Serotype 19A accounted for the largest proportion of nonsusceptible isolates for all antibiotic classes: PEN (69% [59/86]), SXT (62% [61/99]), CTX/CRO (48% [10/21]), TET (45% [9/20]), and ERY (43% [19/44]). Serotype 19A was also responsible for 45% (17/38) of MDR isolates. The proportion of serotype 19A isolates that were nonsusceptible to TET and CTX/CRO increased from the PCV7 era to the PCV13 era: TET (7% [5/72] versus 50% [4/8]; P= 0.004), CTX/CRO (8% [6/72] versus 50% [4/8]; P= 0.007).

CC172 and CC199 accounted for ≥55% of SXT (n= 27 for both) and PEN (n= 28 and 24, respectively) nonsusceptible isolates. Together, ST320 (n= 9) and ST63 (n= 5) were responsible for 70% of TET nonsusceptible isolates. Of the CTX/CRO nonsusceptible isolates, 43% were ST320 (n= 9). Half of the ERY nonsusceptible and MDR isolates were ST320 (n= 9 for both), CC199 (n= 8 and 6, respectively), or ST63 (n= 5 for both).

The proportion of isolates that were nonsusceptible to ERY and TET increased from the PCV7 era to the PCV13 era. Isolates belonging to 3 STs contributed to the majority of ERY and TET nonsusceptibility in the PCV13 era. ST63 (serotype 15A), ST320 (serotype 19A), and ST3280 (serotype 15B/C) combined for 71% (10/14) and 91% (10/11) of ERY and TET nonsusceptible isolates, respectively, in the PCV13 era. Isolates of these 3 STs were also nonsusceptible to PEN and SXT. Additionally, isolates of ST320 were nonsusceptible to CTX/CRO. ST63 and ST320 were circulating in the PCV7 era but ST3280 was not seen in Alaskan children until 2011.

4. Discussion

We have reported on the PCV13 impact on IPD in Alaskans of all ages (Bruce et al., 2015) and here we present changes in the IPD isolate population structure in children <5 years of age. The genetic diversity of pneumococcal isolates in Alaskans <5 years of age has increased in the PCV13 era as compared with the PCV7 era due to a decrease in serotype 19A isolates and an increase in the proportion of isolates expressing NVT serotypes. We have seen an increase in the proportion of isolates nonsusceptible to ERY and TET. This has been partially due to expansion of ST63 (serotype 15A), a highly resistant clone circulating in the PCV7 era. The majority (63%) of isolates from the PCV13 era were associated with STs that were circulating in young children in the PCV7 era leading us to conclude that the population structure change resulted primarily from expansion of existing clones.

Serotype 19A was the most common serotype in our study, making up nearly 30% of isolates and accounting for the largest percentage of antimicrobial nonsusceptible isolates. We previously described the serotype 19A population structure among Alaskans of all ages and in that study CC199, ST172, and CC320 were the most common 19A clones (Rudolph et al., 2013). In this study focusing on Alaskan children <5 years of age, all serotype 19A isolates were one of these clones. CC199, the majority of which were serotype 19A, accounted for nearly 22% of the total number of isolates in the PCV7 era. After routine PCV13 use, CC199 continued to be the most predominant clone; however, its association with serotype 19A changed from 84% of the total CC199 isolates to 50%; the remainder was serotype 15B/C. Although this is not a statistically significant change, it suggests that CC199 19A-IPD is decreasing due to PCV13 vaccine pressure and that persistence of CC199 serotype 15B/C isolates is responsible for the continued circulation of this clone. Other groups have reported increases in serotypes 15B and/or 15C proportions after PCV13 use (Ben-Shimol et al., 2014; Richter et al., 2014; Waight et al., 2015; Metcalf et al., 2016; Mendes et al., 2014). In one study that includes genotype data, CC199 was the most common 15B/C CC (Metcalf et al., 2016). ST172 accounted for 12.2% of isolates in the PCV7 era. After 3 years of PCV13 use, CC172 has nearly disappeared in Alaskan children <5 years of age. The only CC172 isolate identified in the PCV13 era was a serotype 23A found in the first month of PCV13 use. The disappearance of CC172 19A isolates and diminished 19A-associated CC199 is largely responsible for the decreased serotype 19A-PEN, SXT, and ERY nonsusceptible IPD we have seen in the PCV13 era. ST320, a highly resistant serotype 19A clone that was not seen in the United States before 2005 (Pelton et al., 2007), began circulating among Alaskan children in 2007 and continued to be identified in the PCV13 era. This clone is one of three responsible for the majority of the increase in the proportion of ERY and TET isolates seen in the PCV13 era. It is an example of how quickly a ST can spread across geographic areas and suggests that vaccine use is not the only factor involved in serotype and ST changes in a population. It is possible the highly resistant nature of this clone contributes to its continued existence in Alaskan children with IPD.

Serotype 7F made up 12.5% of isolates in our study and was the second most common serotype recovered. All but one serotype 7F isolate belonged to CC191. We previously reported that from 1995 to 2000, CC191 accounted for 2.6% (7/268) of isolates recovered from children <5 years of age (Miernyk et al., 2008) indicating that the large number of serotype 7F isolates identified in this study was due to expansion of this clone circulating before vaccine use. Although the percentages of CC191 found during the PCV7 and PCV13 eras are statistically similar, there has been only 2 CC191 isolates recovered in the PCV13 era. It seems CC191 might be disappearing from this population likely because of PCV13 vaccine pressure. Similar to serotype 19A, many countries around the world have reported changes in serotype 7F proportions after PCV use (Oftadeh et al., 2013; Maraki et al., 2010; Ben-Shimol et al., 2014; Richter et al., 2014; Waight et al., 2015; Mendes et al., 2014; Steens et al., 2013; Dias & Canica, 2007; Demczuk et al., 2013; Kaplan et al., 2013).

Serotype 12F was a major serotype in the PCV13 era where it accounted for over 8% of isolates, all ST218. We previously reported that before PCV7 use (1995–2000), ST218 accounted for 2.3% of IPD isolates from Alaskans of all ages (Miernyk et al., 2008). In this

study it was present in similar proportions in the PCV7 and PCV13 eras. CC433, responsible for 92% of our serotype 22F isolates, was also represented in similar proportions in the PCV7 and PCV13 eras. Metcalf et al. reported their serotype 22F isolates were also almost entirely CC433 and, although they did not report genotyping data, other groups have reported significant increases in serotypes 12F or 22F after PCV13 use (Ben-Shimol et al., 2014; Waight et al., 2015; Metcalf et al., 2016; Demczuk et al., 2013; Levy et al., 2014). Thus, these 2 serotypes, and specifically ST218 and CC433 for Alaska, could cause replacement disease and should be closely monitored as we continue with PCV13 use.

Three NVT serotypes, 15B/C, 15A, and 35B increased in proportion during the PCV13 era. The majority of serotype 15B/C isolates were CC199 and, as described previously, with PCV13 use, its contribution to the total CC199 population could be increasing. CC644 and ST3280 account for the remaining serotype 15B/C isolates. These clones are not increasing in prevalence at this time; however, ST3280 is an MDR clone that is contributing to the increase in the percentage of ERY and TET nonsusceptible isolates we have seen in the PCV13 era. Metcalf et al. reported CC3280 as their second most common 15B/C-associated CC; it was also MDR (Metcalf et al., 2016). An increase in the proportion of ST3280 could have implications in overall nonsusceptibility in Alaska so it should be monitored as we continue with PCV13 use. Serotype 15A isolates were associated with 2 clones, ST63 and ST817. In addition to serotype 15A, the MLST database shows ST63 associated with serotypes 14 and 19A in many parts of the world (Africa, Asia, Australia, and Europe). It is an MDR clone that, in our study, is a contributor to the increase in the proportion of ERY and TET isolates we have seen in the PCV13 era. The proportion of ST63 isolates expanded in the PCV13 era, where they made up almost 10% of all IPD isolates recovered from Alaskan children <5 years of age. After PCV13 use, an increase in serotype 15A prevalence has also been reported in other parts of the United States as well as in Japan and Europe (Richter et al., 2014; Waight et al., 2015; Metcalf et al., 2016; Steens et al., 2013; Janoir et al., 2016; van der Linden et al., 2015; Nakano et al., 2016). It is often an MDR serotype and, as in our study, in the 3 studies reporting genotyping data, CC63 was the most prevalent CC (Metcalf et al., 2016; van der Linden et al., 2015; Nakano et al., 2016). ST817 isolates were only found in the PCV7 era so did not contribute to the serotype 15A increase we are reporting here. This clone likely represents natural fluctuations in the pneumococcal population that are unrelated to vaccine use or antibiotic resistance profile. With the exception of one ST452 isolate, serotype 35B isolates in this study were associated with CC558. Although our CC558 isolates were almost exclusively MDR, an inspection of the MLST database shows that is not universally true. CC558 has been identified throughout the world, generally associated with serotypes 35B or 29. Richter et al. also reported an increase in serotype 35B post-PCV13 in their study of *S. pneumoniae* disease in US citizens of all ages (Richter et al., 2014).

We have identified two potential capsular switching events. In the MLST database all ST644 isolates are reported as serotypes 9V (n= 15), 6B (n= 1), and 19F (n= 1), but in our study ST644 was associated with serotype 15B/C. We first identified this clone in 2009, 8 years after PCV7 introduction; however, it could have been responsible for IPD in older children or adults prior to this. Additionally, we have not investigated the genetic lineage of our 9V, 6B, or 19F isolates prior to 2001 so we do not know if this clone has ever been responsible

for IPD caused by those serotypes in Alaska. However, with what is known from this study, it is possible we have identified a ST644 vaccine-related capsular switching event. ST3934, a serotype 15B/C isolate in our study, was first submitted to the MLST database in 2001 and is exclusively associated with serotype 19A. We first identified this clone in 2002, before PCV13 introduction. It is possible we have identified a ST3934 capsular switching event; however it does not seem to be vaccine-related.

This study's main limitation is the parameters we placed on the IPD isolates we included. We focused this analysis on children <5 years of age as they are the target population for PCVs and we would anticipate changes in the IPD population to be identified more quickly in this age group. This limitation, however, makes it difficult to speculate as to how or when some of the new major clones, such as ST63, CC433, or CC558, were introduced into Alaska. We also do not know whether or not clones such as CC172 or ST817 are truly gone from our population or are still circulating among older children and/or adults. However, despite these limitations, we have been able to identify some serotype and ST trends among Alaskan IPD isolates that should be monitored and studied further.

In conclusion, the population structure of IPD isolates in Alaskans <5 years of age has changed throughout the PCV era. In particular, the proportion of IPD caused by serotype 19A isolates has decreased with PCV13 use; at the same time the proportions of serotype 15B/C isolates, mostly associated with CC199, serotype 35B isolates, associated with CC558, and serotype 15A isolates, associated with ST63, have increased post-PCV13. The proportion of serotype 22F isolates (CC433) and serotype 12F isolates (ST218) has not increased in young Alaskan children but has done so in other populations. ST63, ST320, and ST3280 are responsible for the majority of the increase in the proportion of ERY and TET nonsusceptible isolates collected during the PCV13 era. The pneumococcal population's genetic diversity is increasing as the number of clones associated with NVT serotypes increases. For STs in which we had data prior to 2001, the majority of pneumococcal population changes arose from clonal expansion. As we continue PCV13 use in Alaska, it will be important to maintain a surveillance system that includes serotyping, antimicrobial susceptibility testing and MLST in order to quickly identify any replacement IPD in young children.

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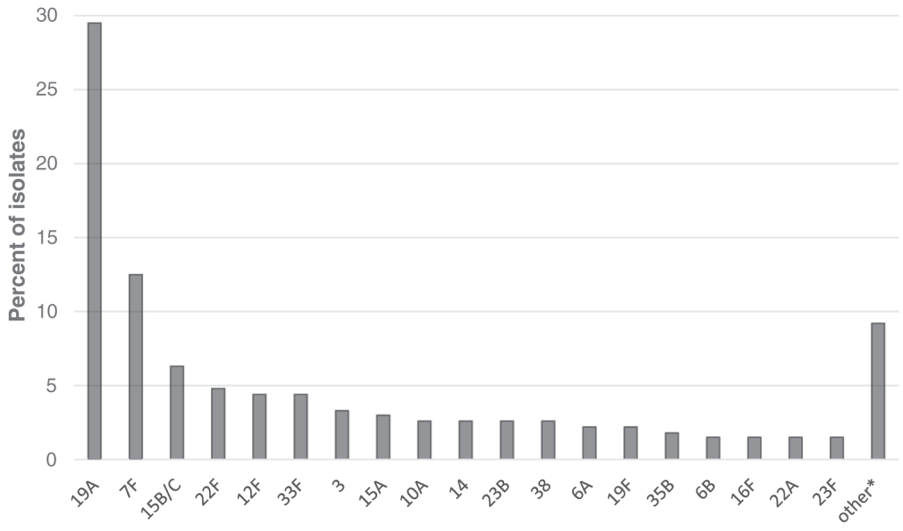

Fig. 1.

Serotypes causing invasive pneumococcal disease in Alaskan children <5 years of age, 2001 to 2013.

*1.1% (serotypes 6C, 8, 35F), 0.7% (serotypes 9N, 9V, 11A, 17F, 18C), 0.4% (serotypes 1, 13, 20, 21, 23A, 31)

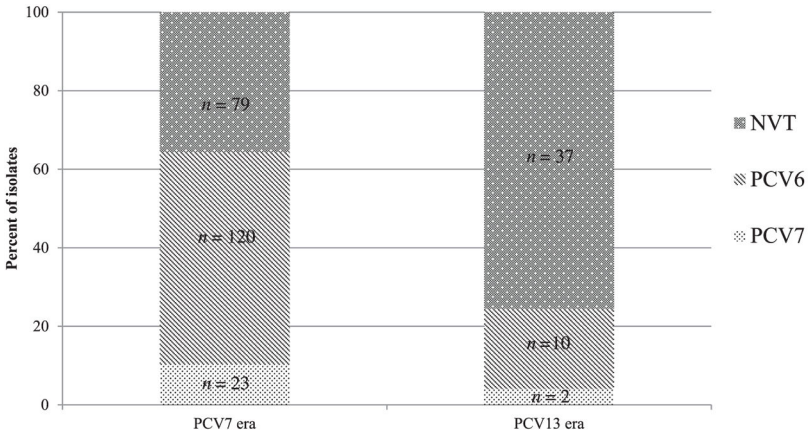

Fig. 2.

Distribution of serotypes causing invasive pneumococcal disease in Alaskan children <5 years of age, 2001 to 2013.

PCV7 = serotypes in the seven-valent pneumococcal conjugate vaccine (4, 6B, 9V, 14, 18C, 19F, 23F); PCV6 = serotypes in the 13-valent pneumococcal conjugate vaccine (PCV13) but not in PCV7 (1, 3, 5, 6A, 7F, 19A); NVT = serotypes not in PCV13

PCV7 era, 2001–3/2010; PCV13 era, 4/2010–2013

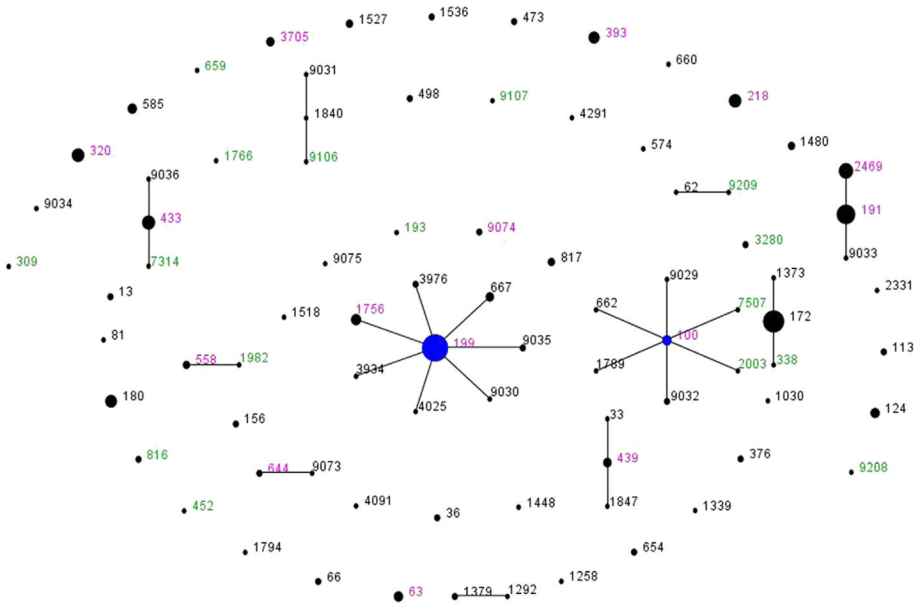

Fig. 3.

Population structure of *S. pneumoniae* causing invasive pneumococcal disease in Alaskan children <5 years of age, 2001 to 2013.

Black (n = 52): Sequence types (STs) identified only during the 7-valent pneumococcal conjugate vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) era, 2001-3/2010

Green (n = 16): STs identified only during the 13-valent pneumococcal conjugate vaccine (PCV7 plus serotypes 1, 3, 5, 6A, 7F, 19A) era, 4/2010-2013

Pink (n = 15): STs identified during both the PCV7 and PCV13 eras

The size of the dots are an indication of the number of isolates associated with that ST

STs included a clonal complex (CC) are connected by a line; if known, the predicted founder of the CC is identified by a blue dot

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Table 1

Clonal complexes (CC) and sequence types (ST) causing invasive pneumococcal disease in Alaskan children <5 years of age stratified by statewide pneumococcal conjugate vaccine use, 2001 to 2013.

CC/ST	Associated serotype(s)	PCV7[a] era 2001–3/2010 N (%)		PCV13[b] era 4/2010–2013 N (%)		Total *N* (%)		Cumulative %	*P* value[c]
CC199	19A, 15B/C	49	22.1%	8	16.3%	57	21.0%	21.0%	NS
CC191	7F	31	14.0%	2	4.1%	33	12.2%	33.2%	NS
CC172	19A, 23A, 23B	27	12.2%	1	2.0%	28	10.3%	43.5%	**0.037**
CC433	22F	9	4.1%	3	6.1%	12	4.4%	48.0%	NS
CC100	33F	9	4.1%	3	6.1%	12	4.4%	52.4%	NS
ST218	12F	5	2.3%	4	8.2%	9	3.3%	55.7%	NS
ST320	19A	5	2.3%	4	8.2%	9	3.3%	59.0%	NS
ST180	3	8	3.6%	0	0.0%	8	3.0%	62.0%	NS
ST393	38	6	2.7%	1	2.0%	7	2.6%	64.6%	NS
CC439	23B, 23F	5	2.3%	1	2.0%	6	2.2%	66.8%	NS
ST63	15A	1	0.5%	4	8.2%	5	1.9%	68.6%	**0.004**
ST124	14	5	2.3%	0	0.0%	5	1.9%	70.5%	NS
ST585	10A	5	2.3%	0	0.0%	5	1.9%	72.3%	NS
CC558	35B	2	0.9%	2	4.1%	4	1.5%	73.8%	NS
ST3705	22A	3	1.4%	1	2.0%	4	1.5%	75.3%	NS
CC644	15B/C	2	0.9%	1	2.0%	3	1.1%	76.4%	NS
ST817	15A	3	1.4%	0	0.0%	3	1.1%	77.5%	NS
CC1379	6C	3	1.4%	0	0.0%	3	1.1%	78.6%	NS
ST1480	8	3	1.4%	0	0.0%	3	1.1%	79.7%	NS
ST1527	12F	3	1.4%	0	0.0%	3	1.1%	80.8%	NS
CC1840	16F	2	0.9%	1	2.0%	3	1.1%	81.9%	NS
ST13	14	2	0.9%	0	0.0%	2	0.7%		
ST36	23F	2	0.9%	0	0.0%	2	0.7%		
ST66	9N	2	0.9%	0	0.0%	2	0.7%		
ST113	18C	2	0.9%	0	0.0%	2	0.7%		
ST156	9V	2	0.9%	0	0.0%	2	0.7%		
ST376	6A	2	0.9%	0	0.0%	2	0.7%		

CC/ST	Associated serotype(s)	PCV7[a] era 2001–3/2010 N (%)		PCV13[b] era 4/2010–2013 N (%)		Total *N* (%)		Cumulative %	*P* value[c]
ST473	6A	2	0.9%	0	0.0%	2	0.7%		
ST498	35F	2	0.9%	0	0.0%	2	0.7%		
ST654	19F	2	0.9%	0	0.0%	2	0.7%		
ST816	10A	0	0.0%	2	4.1%	2	0.7%		
ST1536	6B	2	0.9%	0	0.0%	2	0.7%		
ST3280	15B/C	0	0.0%	2	4.1%	2	0.7%		
CC62	11A	1	0.5%	1	2.0%	2	0.7%		
ST9074	19F	1	0.5%	1	2.0%	2	0.7%		
ST81	23F	1	0.5%	0	0.0%	1	0.4%		
ST193	21	0	0.0%	1	2.0%	1	0.4%		
ST309	19F	0	0.0%	1	2.0%	1	0.4%		
ST452	35B	0	0.0%	1	2.0%	1	0.4%		
ST574	13	1	0.5%	0	0.0%	1	0.4%		
ST659	16F	0	0.0%	1	2.0%	1	0.4%		
ST660	6A	1	0.5%	0	0.0%	1	0.4%		
ST1030	20	1	0.5%	0	0.0%	1	0.4%		
ST1258	19F	1	0.5%	0	0.0%	1	0.4%		
ST1339	6A	1	0.5%	0	0.0%	1	0.4%		
ST1448	23B	1	0.5%	0	0.0%	1	0.4%		
ST1518	6B	1	0.5%	0	0.0%	1	0.4%		
ST1766	31	0	0.0%	1	2.0%	1	0.4%		
ST1794	17F	1	0.5%	0	0.0%	1	0.4%		
ST2331	7F	1	0.5%	0	0.0%	1	0.4%		
ST4091	3	1	0.5%	0	0.0%	1	0.4%		
ST4291	17F	1	0.5%	0	0.0%	1	0.4%		
ST9034	1	1	0.5%	0	0.0%	1	0.4%		
ST9075	6B	1	0.5%	0	0.0%	1	0.4%		
ST9107	35F	0	0.0%	1	2.0%	1	0.4%		
ST9208	22F	0	0.0%	1	2.0%	1	0.4%		
Total		222		49		271			
Simpson’s diversity		0.91		0.95		0.92			**0.022**

[a] 7-valent pneumococcal conjugate vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F).

[b] 13-valent pneumococcal conjugate vaccine (PCV7 plus serotypes 1, 3, 5, 6A, 7F, 19A).

[c] for CC/ST with $n \geq 3$.

Table 2

Proportion of antimicrobial nonsusceptible (NS) isolates causing invasive pneumococcal disease in Alaskan children <5 years of age, 2001 to 2013.

Antibiotic[a]	PCV7[b] era 2001–3/2010 N (%)	PCV13[c] era 4/2010–2013 N (%)	Total 2001–2013 N (%)	*P* value
SXT NS	85 (38%)	14 (29%)	99 (37%)	0.201
PEN NS	70 (32%)	16 (33%)	86 (32%)	0.879
ERY NS	30 (14%)	14 (29%)	44 (16%)	**0.010**
CTX/CRO NS	15 (7%)	6 (12%)	21 (8%)	0.193
TET NS	9 (4%)	11 (22%)	20 (7%)	<**0.001**
MDR	27 (12%)	11 (22%)	38 (14%)	0.061

[a] NS = nonsusceptible.

[b] 7-valent pneumococcal conjugate vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F).

[c] 13-valent pneumococcal conjugate vaccine (PCV7 plus serotypes 1, 3, 5, 6A, 7F, and 19A).